

ELECTROCHEMICAL DETECTION OF DOPAMINE IN PRESENCE OF URIC ACID USING A ZIRCONIA MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT

A Sensitive and selective electrochemical sensor was developed by modifying carbon paste electrode (MCPE) with Zirconia for the simultaneous determination of dopamine (DA) in the presence of excess concentration of uric acid (UA) in 0.1 M phosphate buffer solution (PBS) at pH 7.0 with a scan rate of 50 mVs⁻¹. Cyclic and differential pulse voltammetric techniques were carried out to study the electrochemical properties of modified electrode. The parameters including carbon paste composition, scan rate, electrode potential and pH were studied. The oxidation peak potential of UA exhibited a negative shift due to the electrostatic repulsions of anionic species with zirconia modified carbon paste electrode (ZMCPE). A fourfold enhancement in oxidation peak current was observed with ZMCPE when compared to the bare carbon paste electrode (BCPE). The anodic oxidation peak current (I_{pa}) Vs scan rate (v) was linear with a correlation co-efficient of 0.9939 which indicates that it is a diffusion controlled reaction. The concentration of UA ranges from 0.01 μ M to 0.2mM with a correlation coefficient of 0.9975 and the detection limit was found to be 5.1×10^{-7} M. The detection limit of UA and DA were 0.01 μ M and 0.04 μ M with correlation coefficients of 0.97028 and 0.97103 respectively. This modified electrode showed an excellent sensitivity, selectivity, stability, fast response and reliability for the voltammetric determination of DA in the presence of UA and the same was satisfactorily used for the determination of UA in human serum and urine samples.

KEYWORDS: Uric Acid (UA), Dopamine (DA), Zirconia (ZrO_2), Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV)

INTRODUCTION

In recent years, considerable interest has been evinced in novel electro chemical sensors in design, fabrication and their applications in chemical and biological samples [1]. Particularly the developments of voltammetric sensors have a several distinct advantages at lower over potentials. It increases the reaction rate and sensitivity that can further improve the selectivity [2-5]. Uric Acid (UA) is a small electro active biomolecule and is the final end product of purine metabolism in the human body fluids [6]. Uric acid (2,6,8-trihydroxypurine) is found in high concentrations in meat products especially in the internal organs of liver and kidney [7-8]. In healthy persons, the concentration of UA in urine is around 2mM and in the blood it is in the range of 120 μ M to 450 μ M. Normally, healthy human beings excrete about 400 to 700 mg through urine per day [9]. UA concentration variations are associated with the altered metabolism of purines which are related to numerous mal functions and physiological disorders [10]. High levels of UA causes hyperuricemia, excess serum accumulation of UA in the blood can lead to arthritis (gout) [11]. The low concentration of UA leads to hyperuricemia and other diseases such as Lesch-Nyhan syndrome and heavy hepatitis [12]. UA is also a marker for the renal failure as well as toxicity. Therefore, its determination in body fluids is necessary for the treatment of diseases.

Dopamine (DA) is a unique neurotransmitter compound extensively distributed in the brain for message transfer in the mammalian central nervous system. Low levels of DA can cause neurological disorders such as, Parkinson's disease, schizophrenia and HIV infection [13,14]. Therefore, simultaneous determination of DA and UA has attracted many researchers. Several simple analytical methods with simple procedures are essential because they serve as markers for the detection of above diseases. Hence, it is very important for the development of simple, rapid and selective methods for their simultaneous determination in clinical analysis. To achieve this purpose, several modified electrodes are used for the determination of DA in the presence of UA [15,16].

UA and DA are the electro active compounds that can be determined by using their oxidation potentials. Due to similar oxidation potentials of UA and DA, it is rather difficult to resolve the corresponding peaks by using unmodified electrodes, which result in the overlapped voltammetric responses making their discrimination very difficult. However, some efforts have been made to develop a new electrochemical sensor for the simultaneous determination of UA and DA by using different methods such as metal oxides [17,18] clays [19], polyoxometalates [20], zeolites [21], choline and acetylcholine-modified glassy carbon electrode [22], oracetblue modified electrode [23], caffeic acid (3,4-dihydroxy cinnamic acid) [24], didodecyl-bis-methyl ammonium bromide modified electrode [25], tetrabromo-p-benzoquinone modified electrode [26]. To our knowledge there was no report on the inorganic metal oxide of zirconia used as an electrode material. Murray Co-workers [27-29] and Burt-et al [30] were the early groups to use the inorganic metal oxides as electrode material because of their properties like electronic conductivity and excellent catalytic characteristics. These inorganic metal oxides have chains on the metal centers with different oxidation states and have distinct advantages like stability, reproducibility, more active sites for the electrochemical deposition and strong adherence of the electrode in the field of chemically modified electrodes.

Zirconia is an inorganic metal oxide which possesses efficient properties like good chemical inertness, thermal stability, more hardness, good resistance [31-33] and low-toxicity even at extreme conditions. It has an additional advantage as electrode material due to its wide band gap energy (5.78ev) [34] and the absence of photo catalytic activity under solar light radiation. Accordingly, the Zirconia modified carbon paste electrode (ZMCPE) is a good chemical sensor for the selective and sensitive detection of UA and DA in the analyses of chemical and biological samples.

EXPERIMENTAL PART

Materials and Stock Solution

Analytical grade Uric acid (UA), sodium hydrogen orthophosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), Sodium hydroxide (NaOH), silicon oil were procured from Himedia chemicals. Fine graphite powder (particle<20 size μm) was purchased from Sigma-Aldrich. Uric acid stock solution (25mM) was prepared in 0.1M sodium hydroxide; Phosphate buffer (pH 7.0) was prepared with 0.2M NaH_2PO_4 and Na_2HPO_4 solution with distilled water.

Instrumentation

All voltammetric studies were done with a CH-Instrument Model Number. CHI 610D Electrochemical work station connected to a personal computer. A conventional three electrode cell was employed throughout the experiments, with bare and ZrO_2 modified carbon paste electrodes (home made cavity of 3.0 mm diameter) as a working electrode, Ag/AgCl electrode as a reference electrode and a platinum wire as a counter electrode.

Preparation of Bare Carbon Paste Electrode

The carbon paste electrode was prepared by hand mixing of 70% graphite powder and 30% of silicon oil using agate mortar for 40 min to get a homogeneous paste [35]. Then the prepared carbon paste was incorporated into 3.0 mm diameter of Teflon cavity current collector and polished using the soft paper before using.

Preparation of Modified Carbon Paste Electrode

Carbon paste electrode (CPE) was modified by taking different weights of zirconia (modifier-ZrO₂) (5,15,25,35 and 45mg) in silicon oil (30%) and graphite powder (70%). This mixture was thoroughly mixed in an agate mortar for about 40 min and packed into a 3.0 mm diameter of Teflon cavity current collector and polished on a piece of soft paper before measurement.

RESULTS AND DISCUSSIONS

Electrochemical Investigation of Uric Acid at the ZrO₂ MCPE

The concentration effect of Zirconia in the carbon paste electrode was studied with 0.1 M phosphate buffer solution of pH 7.0 in 1 mM UA by using cyclic voltammetry method. The electrochemical responses of 5,15,25,35 and 45 mg of ZrO₂ are shown in figure1. The modified carbon paste electrode with 25mg of Zirconia showed a highest anodic peak current and lower oxidation potential when compared to the bare CPE. The substantial increase in the peak current and decrement in peak potential were observed due to the irreversibility of the electron transfer process, which suggests that it is an efficient oxidation reaction of UA at the ZMCPE.

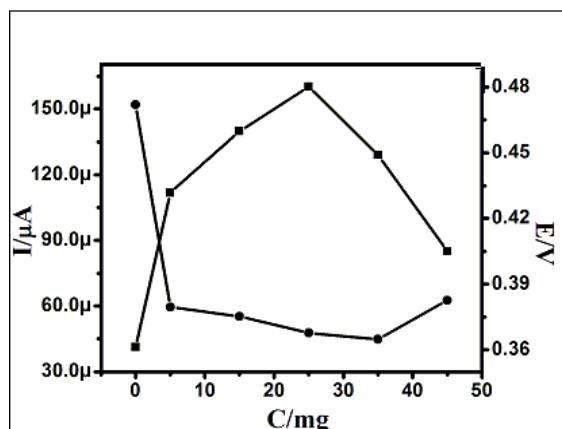


Figure 1: Cyclic Voltammograms of UA at the Bare Carbon Electrode and ZrO₂ Modified Electrode in 0.1 M Phosphate Buffer at a Scan Rate of 50 mV/s

Cyclic Voltammetric Study of UA on the MCPE

Cyclic voltammetry experiments were carried out in the presence of 1 mM UA at 0.1 M phosphate buffer solution (pH 7.0) with a scan rate of 50 mVs⁻¹. The cyclic voltammogram obtained for Zirconia modified carbon paste electrode (ZMCPE) (curve-a) and bare carbon paste electrode (curve-b) are shown in figure-2. The modified electrode showed a good increment in anodic peak current (I_{pa}) and decrement in the anodic peak potential (E_{pa}) with a difference of peak potential of 0.1050 V when compared with the bare carbon paste electrode. There was a 4 folds enhancement in anodic peak current with the reduction in oxidation peak potential of ZMCPE and this indicates the electro-catalytic nature of ZMCPE.

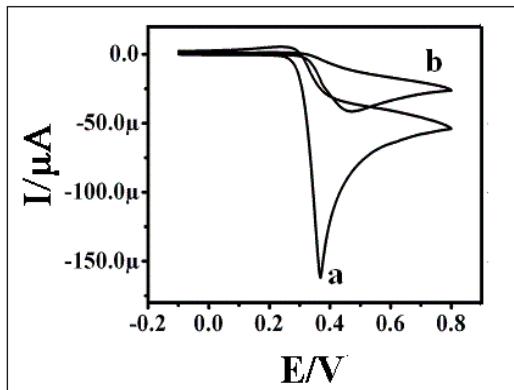


Figure 2: The Relation between Anodic Peak Current (I_{pa}) vs Concentration of ZrO₂ in Carbon Paste Electrode

Effect of Scan Rate

The effect of scan rate for 1 mM UA in 0.1 M PBS at pH 7.0 was studied by cyclic voltammetry with ZMCPE. When the scan rate increases, the oxidation peak current was also increased gradually, due to the transfer of electrons between UA and the ZMCPE. In the range of 50 to 450 mV/s⁻¹ the oxidation peak currents were proportional to the square root of scan rate according to the Randles-sevick's equation [35] with a correlation coefficient of (R²) 0.9939, as shown in figure-3 indicating the electrode reaction as a diffusion controlled process.

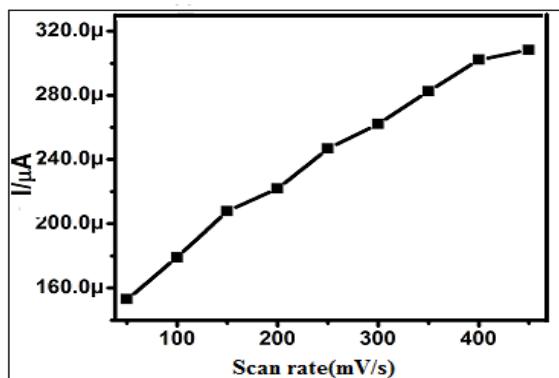


Figure 3: Plot for Anodic Peak Current as Function of Scan Rate (50mV/s to 450mV/s) for 1mM UA at ZMCPE

Effect of pH

Different experiments were carried out in various supporting electrolytes (phosphate and acetate) buffers at different pH values in order to assess their impact on the monitored electro analytical signal. The results with ZMCPE, sensitivity accompanied with sharper response was obtained with phosphate buffer solution (PBS) at physiological pH. The study was carried out in the pH range 5.5–8.0 in phosphate buffers at a target concentration of 1 mM UA solution. With increase in pH, the peak potential was shifted towards negative direction due to the release of proton. This shows that the protons are directly participated in the oxidation process [36]. The maximum peak current was obtained at pH 7.0. Therefore, a pH of 7.0 was selected for all subsequent electrochemical analyses of UA. The effect of pH Vs peak currents and peak potentials at a scan rate of 50mV/s is shown in figure 4.

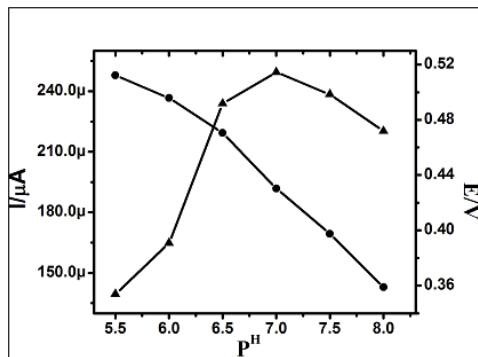


Figure 4: Effect of pH on the Electrochemical Behavior of 2.5 mM of UA at ZMCPE in 0.1M PBS with Different pH Conditions

Effect of Concentration

Differential pulse voltammetry was used in the trace level detection and interference studies of different analytes. DPV was used to study the concentration effect of UA by varying from 0.01 μ M to 0.2 mM at the ZMCPE in 0.1M PBS at pH 7.0 and the resulting voltammograms are shown in figure-5. The graph plotted between anodic peak current Vs concentration of UA showed two linear relationships ranging from 0.01 μ M to 0.3 μ M and 0.4 μ M to 0.2 mM with a correlation coefficients (R^2) of 0.997 and 0.995 respectively. The developed analytical method was validated with respect to parameters such as limit of quantification (LOQ), limit of detection (LOD). The detection limit was calculated by using the equation $LOD = 3S_b/S$, where S_b is standard deviation of intercept and S is slope of the calibration graph. The detection limit was found to be 0.05 μ M. The quantification limit was examined by the equation $LOQ = 10S_b/S$. The lower limit of quantification for the standard solution was found to be 0.186 μ M.

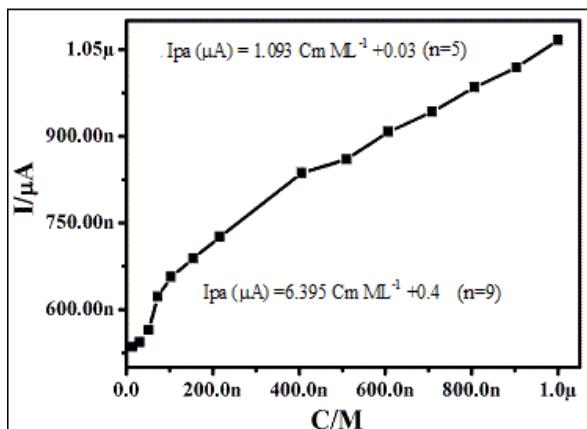


Figure 5: DPV Curves of UA at Various Concentrations in 0.1M PBS in the Presence of ZMCPE

Simultaneous Detection of DA in the Presence of UA at ZrO_2 MCPE

The main goal of our present study is to determine the sensitivity and selectivity of DA in the presence of UA. The cyclic voltammograms were taken for the mixture of DA and UA in 0.1 M PBS at pH 7.0 with BCPE and ZMCPE at a scan rate of 50 mVs⁻¹. The peak potentials of DA and UA were well resolved with ZMCPE but not with the BCPE as shown in figure-6. Electro-catalytic activity of the ZMCPE showed a good enhancement in the oxidation peak current of each analyte with a well resolved peak potential separation of 0.260 V between DA and UA, at a physiological pH, UA

exists as anion and DA exists as cation. Figure 7 shows the Differential Pulse Voltammograms obtained due to the change in concentration of DA from 1mM to 6.25 mM and UA from 1.04 mM to 8.03 mM in 0.1 M PBS at pH 7.0 and anodic peak current was linearly proportional to the concentration of UA and DA with a co-relation co-efficients (R^2) of 0.97028 and 0.97103 respectively as shown in figure-8. Thus, the modified electrode can selectively detect the DA in presence of UA with high sensitivity, low detection limit and good electro catalytic activity.

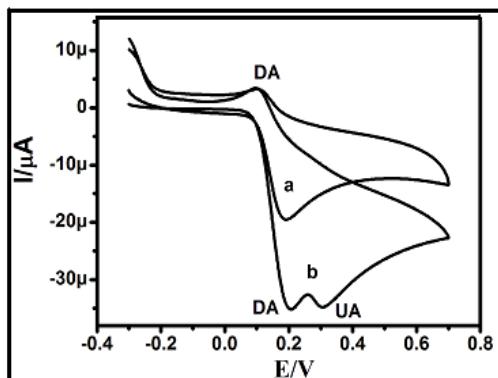


Figure 6: Cyclic Voltammograms of 2.5mM UA and DA Mixture in pH 7.0 PBS at (a) Bare and (b) at ZMCPE

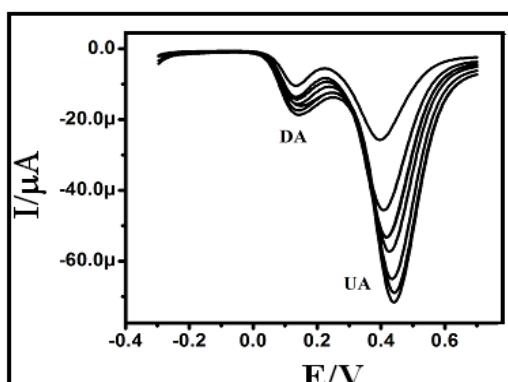


Figure 7: Differential Pulse Voltammograms for Simultaneous Determination of 2.5 mM UA and 2.5mM DA in pH 7.0 with the Scan Rate of 50 mV/s

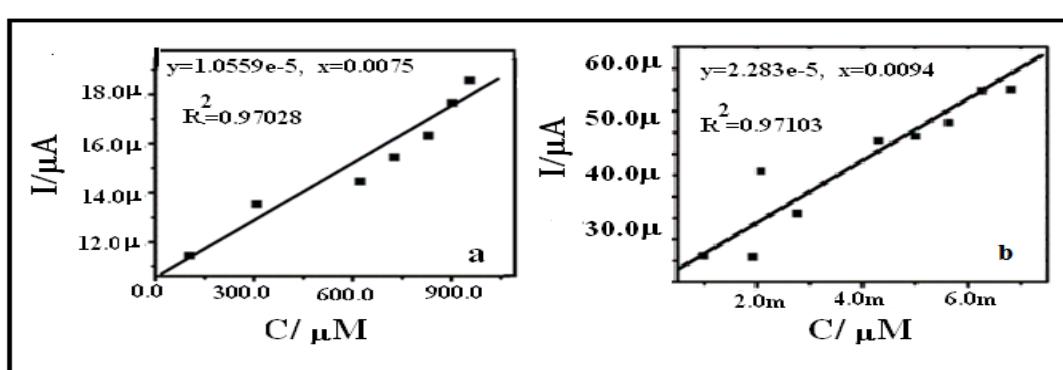


Figure 8: (a) Effect of DA Concentration on the Electrochemical Behavior of UA at the ZrO_2 MCPE in pH 7.0 in PBS with the Scan Rate 50 mV/s. DA Concentration from 1.04 mM to 8.03 mM. (b) Plot of Peak Current vs UA Concentration from 1 mM to 6.81 mM in pH 7.0 in PBS with the Scan Rate 50 mV/s

Real Sample Analysis

Zirconia modified carbon paste electrode was used for the quantitative determination of UA in human urine and

serum samples (obtained from the Health Centre, Sri Venkateswara University, Tirupati, Andhra Pradesh, India). In the analysis: 2 ml of human serum sample without any pretreatment was diluted to 100 mL with pH 7.0 phosphate buffer. The fresh urine samples were diluted, before electrochemical measurements. 10 μ L urine sample was diluted 10 times and then the diluted urine samples were analyzed by the same differential pulse voltammetric procedure as the UA standard solution.

Table 1 shows the quantification results of the uric acid in human serum and urine samples. The analytical method was validated for the recovery and relative standard deviation.

Table 1: Recovery and Determination of Uric Acid in Human Serum and Urine Samples (N=5)

Samples	Spiked(Mm)	Founded(Mm)	Recovery (%)	RSD (%)
Human Serum	0.1	0.09	90%	5.5
	0.2	0.17	92%	2.4
	0.3	0.40	101%	1.4
Human Urine	0.1	0.09	90%	5.5
	0.2	0.17	92%	3.2
	0.3	0.42	104%	1.8

CONCLUSIONS

The present work demonstrates that the ZMCPE can be used for the detection of DA in the presence of UA in real samples. The modified CPE shows an excellent reproducibility, good selectivity, low detection limit, sensitivity and stability for the simultaneous determination of electro catalytic oxidation of UA and DA at physiological pH 7.0 in PBS. The proposed method has been applied to describe the qualitative estimation of UA in human serum and urine sample with satisfactory results. Hence the developed and validated method is well suited for the analysis of UA in real samples.

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